Marked Up Page --

with the appropriate sized insert in the correct orientation relative to the T7 promoter was identified by separate digestion with NheI, NcoI or XhoI. By subcloning fragment in the NheI site of pET9d DN, the putative precursor polypeptide was joined to the ATG codon downstream from the T7 promoter and bacterial Shine & Dalgarno sequence provided by pET9d DN. This NheI site changed the nucleotide sequence of the putative N-terminus of the precursor protein from SEQ ID NO: 57 ~~ATG GCG CTC CGC~~ to SEQ ID NO: 58 ~~ATG GCT AGC CTC CGC,~~ which changed the encoded SEQ ID NO: 59 ~~Met-Ala-Leu-Arg~~ amino acid sequence to SEQ ID NO: 60 ~~Met-Ala-Ser-Leu-Arg.~~ The insert of this clone was sequenced entirely and the clone was assigned number pDAB432D

The precursor maize Δ9 desaturase was expressed in *E. coli* BL21(DE3) (Novagen Inc., Madison, WI). For small scale expression, 1 µg of plasmid pDAB432D was transformed into 200 µL $CaCl_2$ competent cells and plated on two LB plates containing kanamycin at 25 µg/mL. Following overnight incubation at 37° C colonies were scraped off the plate and resuspended in 10 mL of LB Broth containing kanamycin (50 µg/mL) and isopropyl-β-D-thiogalactoside (IPTG) at 1.0 mM. Cells were allowed to express proteins for 3 hr during vigorous shaking at 37° C. Cells were harvested by centrifugation at 3000 rpm for 10 min at 4° C. The cell pellet was frozen and thawed twice in dry ice-ethanol to improve cell lysis. Next, the cell pellet was resuspended in 1.0 mL of lysis buffer (10 mM Tris HCl pH 8.0, 1.0 mM EDTA, 150 mM NaCl, 0.1% Triton X 100, 100 µg/mL DNaseI, 100 µg/mL RNaseH, 1.0 mg/mL lysozyme) and incubated at 37° C until it was no longer viscous. Soluble proteins were separated from aggregated denatured proteins by centrifugation at 4° C for 10 min. The insoluble pellet was resuspended in about 300 µL of the above lysis buffer. Both fractions had an approximate final volume of 0.5 mL.

with the appropriate sized insert in the correct orientation relative to the T7 promoter was identified by separate digestion with NheI, NcoI or XhoI. By subcloning fragment in the NheI site of pET9d DN, the putative precursor polypeptide was joined to the ATG codon downstream from the T7 promoter and bacterial Shine & Dalgarno sequence provided by pET9d DN. This NheI site changed the nucleotide sequence of the putative N-terminus of the precursor protein from SEQ ID NO:57 to SEQ ID NO:58, which changed the encoded SEQ ID NO:59 amino acid sequence to SEQ ID NO:60. The insert of this clone was sequenced entirely and the clone was assigned number pDAB432D.

The precursor maize Δ9 desaturase was expressed in *E. coli* BL21(DE3) (Novagen Inc., Madison, WI). For small scale expression, 1 μg of plasmid pDAB432D was transformed into 200 μL $CaCl_2$ competent cells and plated on two LB plates containing kanamycin at 25 μg/mL. Following overnight incubation at 37° C colonies were scraped off the plate and resuspended in 10 mL of LB Broth containing kanamycin (50 μg/mL) and isopropyl-β-D-thiogalactoside (IPTG) at 1.0 mM. Cells were allowed to express proteins for 3 hr during vigorous shaking at 37° C. Cells were harvested by centrifugation at 3000 rpm for 10 min at 4° C. The cell pellet was frozen and thawed twice in dry ice-ethanol to improve cell lysis. Next, the cell pellet was resuspended in 1.0 mL of lysis buffer (10 mM Tris HCl pH 8.0, 1.0 mM EDTA, 150 mM NaCl, 0.1% Triton X 100, 100 μg/mL DNaseI, 100 μg/mL RNaseH, 1.0 mg/mL lysozyme) and incubated at 37° C

until it was no longer viscous. Soluble proteins were separated from aggregated denatured proteins by centrifugation at 4° C for 10 min. The insoluble pellet was resuspended in about 300 μL of the above lysis buffer. Both fractions had an approximate final volume of 0.5 mL.

with the appropriate sized insert in the correct orientation relative to the T7 promoter was identified by separate digestion with NheI, NcoI or XhoI. By subcloning fragment in the NheI site of pET9d DN, the putative precursor polypeptide was joined to the ATG codon downstream from the T7 promoter and bacterial Shine & Dalgarno sequence provided by pET9d DN. This NheI site changed the nucleotide sequence of the putative N-terminus of the precursor protein from ~~ATG GCG CTC CGC to~~ SEQ ID NO:57 to SEQ ID NO:58 ~~ATG GCT AGC CTC CGC~~, which changed the encoded ~~Met-Ala-Leu-Arg~~ SEQ ID NO:59 amino acid sequence to ~~Met-Ala-Ser-Leu-Arg~~ SEQ ID NO:60. The insert of this clone was sequenced entirely and the clone was assigned number pDAB432D.

The precursor maize Δ9 desaturase was expressed in *E. coli* BL21(DE3) (Novagen Inc., Madison, WI). For small scale expression, 1 µg of plasmid pDAB432D was transformed into 200 µL $CaCl_2$ competent cells and plated on two LB plates containing kanamycin at 25 µg/mL. Following overnight incubation at 37° C colonies were scraped off the plate and resuspended in 10 mL of LB Broth containing kanamycin (50 µg/mL) and isopropyl-β-D-thiogalactoside (IPTG) at 1.0 mM. Cells were allowed to express proteins for 3 hr during vigorous shaking at 37° C. Cells were harvested by centrifugation at 3000 rpm for 10 min at 4° C. The cell pellet was frozen and thawed twice in dry ice-ethanol to improve cell lysis. Next, the cell pellet was resuspended in 1.0 mL of lysis buffer (10 mM Tris HCl pH 8.0, 1.0 mM EDTA, 150 mM NaCl, 0.1% Triton X 100, 100 µg/mL DNaseI, 100 µg/mL RNaseH, 1.0 mg/mL lysozyme) and incubated at 37° C until it was no longer viscous. Soluble proteins were separated from aggregated denatured proteins by centrifugation at 4° C for 10 min. The insoluble pellet was resuspended in about 300 µL of the above lysis buffer. Both fractions had an approximate final volume of 0.5 mL.